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X-Linked Neurodegenerative Syndrome With Congenital Ataxia, Late-Onset Progressive Myoclonic Encephalopathy and Selective Macular Degeneration, Linked to Xp22.33-pter

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Linkage analysis was performed in a previously described family segregating for an X-linked progressive neurological disorder [Bertini et al., 1992]. In three generations, the disease was inherited from the mothers in seven affected males (Fig. 1). Five had severe congenital hypotonia and died during the first year of life. Two other boys (maternal cousins) were found to have severe congenital ataxia, late-onset progressive myoclonic encephalopathy, and selective macular degeneration; brain CT-scan showed moderate cerebellar vermis hypoplasia. Linkage analysis was carried out in 12 informative relatives using 35 microsatellite markers (Généthon) evenly distributed on the X chromosome. A multipoint analysis showed a significant linkage ($Z > 2$) between the disease and three markers in the Xp22.33 region: DYS403 ($Z = 2.37$, $\theta = 0$) which maps in the pseudoautosomal region, DXS7099 ($Z = 2.45$, $\theta = 0$), and DXS7100 ($Z = 2.48$, $\theta = 0$). Further linkage analysis with more telomeric markers will refine the location of this severe X-linked encephalopathy.

KEY WORDS: congenital ataxia, macular degeneration, myoclonic encephalopathy, mental retardation, X-linked recessive, genetic mapping

INTRODUCTION

Almost 60 different syndromic forms of X-linked mental retardation (XLMR) were listed by Neri et al. [1994] in a recent update inventory. Bertini et al. [1992] reported an Italian family segregating for a disorder of congenital ataxia, progressive myoclonic encephalopathy (PME) and macular degeneration. To our knowledge, this condition does not fit one of the known X-linked conditions and seems to be an original and rare syndrome affecting multiple structures of the central nervous system: cerebral cortex, cerebellum, and retinis. Extensive clinical, neuroradiological, biochemical, and immunological investigations were performed in order to understand this new X-linked neurological syndrome [Bertini et al., 1992]. Nevertheless, the pathophysiology remains unknown and no biological marker is available to direct further investigations; therefore positional cloning and gene isolation are alternative approaches for further understanding of this rare and original condition.

In this paper we have performed a linkage analysis, using highly polymorphic microsatellites developed at Généthon. We excluded almost the whole X chromosome and found significant linkage with markers in the Xp22.33 region.

CLINICAL REPORTS

Detailed clinical data of this severe familial encephalopathy were described previously by Bertini et al. [1992]. In an Italian family, seven affected males of the same kindred were observed in three generations, suggesting X linked inheritance (Fig. 1).

Patient II-6 had neonatal generalized hypotonia and died at age one year; patients III-7, III-8, III-9, and IV-3, died before the age of 1 year of bronchopneumonia and were reported to have delayed psychomotor development and marked generalized hypotonia since birth. Obligatory carrier females (II-2, III-2, and III-17) appeared neurologically normal.

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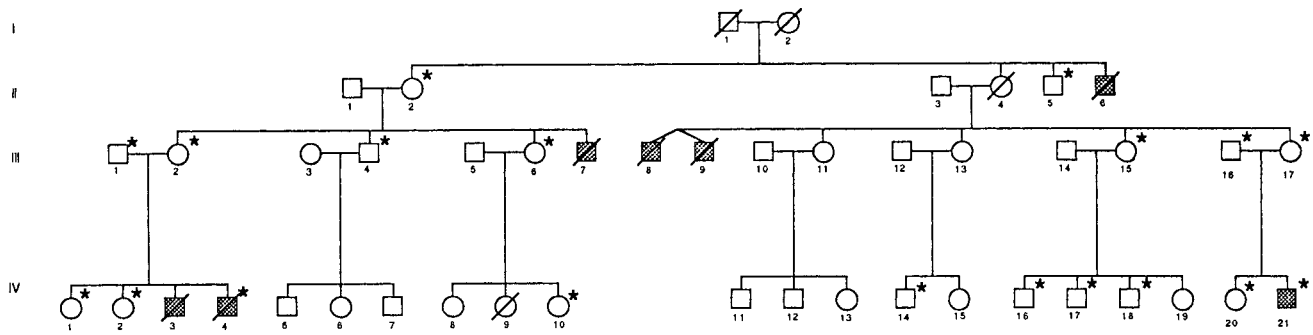

Fig. 1. Pedigree of the family affected with the X-linked progressive neurological syndrome. (▨), Affected males; *, DNA collected.

Two other boys (maternal cousins IV-4 and IV-21) were thoroughly examined and developed quite similar manifestations including severe congenital ataxia, late-onset progressive myoclonic encephalopathy, and macular degeneration: they had neonatal generalized hypotonia and a weak suck, delayed motor development with unsteadiness of head and trunk, and walked with support at 4 years. Language was poor and dysarthric. Recurrent severe episodes of bronchopneumonia and generalized eczema had occurred between 3 months and 4 years. Seizures started at age 5 as daily recurring head drop-attacks, tonic seizures and myoclonic jerks. In the following years, the clinical course was characterized by intractable seizures, motor and mental deterioration and blindness at age 10. Fundoscopy demonstrated a selective bilateral macular degeneration.

Laboratory data ruled out lysosomal diseases, lipofuscinosis, organic acidurias, aminoacidopathies, and biotinidase deficiency. High resolution cytogenetic analysis was normal. Brain CT-scan showed moderate cerebellar vermis hypoplasia without cortical atrophy.

Patient IV-4 died recently at age 15; neuropathological examination of frontal cortex showed neither neuronal loss nor storage products.

MATERIALS AND METHODS

Blood was collected from the relatives indicated on the pedigree (Fig. 1). DNA was extracted by standard procedures. Linkage analysis was carried out in 12 informative family members: 2 affected males, 3 carrier females, and 7 healthy siblings. 35 highly polymorphic microsatellites from Genethon [Gyapay et al., 1994] were used for genotyping: 28 markers evenly distributed on the X chromosome were tested for primary mapping (DXS1193, DXS1227, DXS1205, DXS1062, DXS1001, DXS1220, DXS1059, DXS1210, DXS1230, DXS1231, DXS1106, DXS990, DXS1217, DXS1196, DXS453, DXS1275, DXS991, DXS1204, DXS255, DXS1003, DXS993, DXS1068, DXS1214, DXS992, DXS1226, DXS999, DXS1223, and DXS996). Then, seven further markers located to Xp22.33-pter including some from the latest version of the Généthon's genetic map [Dib et al., 1996] were tested to precise the locus interval (DXS1060, DXS7107, DXS7100, DXS1228, DXS7099, DXS1233, and DYS403). Two-point disease-to-marker linkage analysis was done using MLINK of the LINKAGE package, version 5.2 [Lathrop and Lalouel, 1984], and multipoint analysis was conducted using LINKMAP of the FASTLINK package, version 2.3P [Cottingham et al., 1993]; the mutation rate, gene frequency and penetrance were set at 0.00, 0.0005, and 1, respectively. The genetic distances used for multipoint analysis were as described [Gyapay et al., 1994; Dib et al., 1996] and are represented in Figure 2.

RESULTS

Initial genotyping with 28 (21 informative) markers spanning the X chromosome excluded almost the whole chromosome and indicated potential linkage between the disease and marker DXS996, in Xp22.32 (two-point lod score, $Z = 0.98$ for $\theta = 0.1$) (Table I). Linkage analysis with seven other markers in the Xp22.33 region also exhibited possible linkage to the disease: five informative markers (DXS7107, DXS7100, DXS7099, DXS1233, and DYS403) did not show any recombination with the disease, with a positive but non significant two-point lod score (Table I). A recombination event at DXS1060 ($Z = 0.84$, $\theta = 0.1$) defined the centromeric limit to the disease locus (Fig. 2).

A five-point linkage analysis, including the disease locus, DYS403, DXS7099, DXS7100, and DXS1060, showed significant generalized lod scores [Ott, 1991], commonly called multipoint lod scores Z, which were above the critical value $Z = 2$ for the three distal markers DYS403 ($Z = 2.37$, $\theta = 0$), DXS7099 ($Z = 2.45$, $\theta = 0$), and DXS7100 ($Z = 2.48$, $\theta = 0$); lod score values Z are location score divided by 4.6. Accurate disease assignment within the Xp22.33 region was not possible because there were no informative crossing over events.

These data suggest that the gene causing this severe X-linked encephalopathy maps to the distal short arm in Xp22.3-pter, at the edge or within the pseudoautosomal region (Fig. 2).

DISCUSSION

Patients in this family have congenital ataxia, progressive myoclonic encephalopathy (PME), and macular degeneration. As far as we know, this association is quite unusual and X-linked inheritance has not

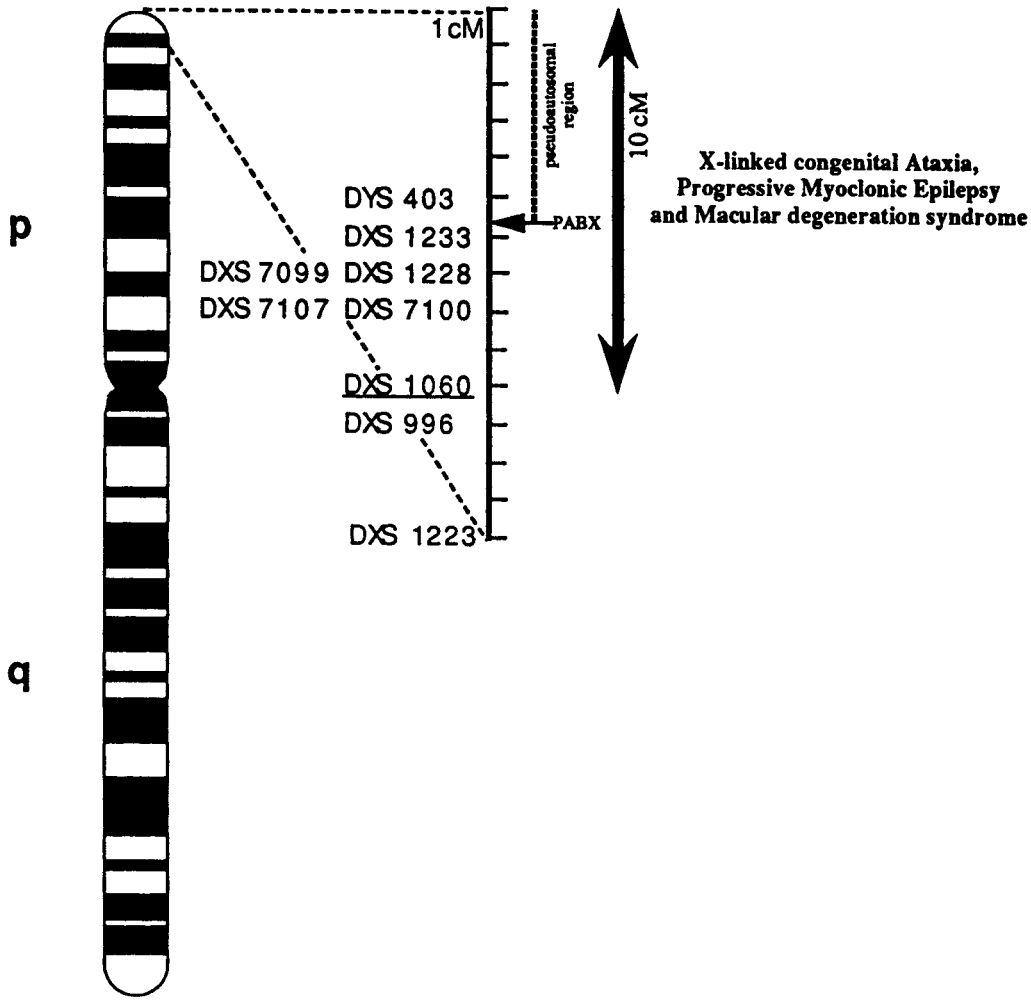

Fig. 2. Ideogram of the X chromosome showing the locus of the X-linked neurological syndrome in the present family. Markers order and the genetic distances are represented as published by Gyapay et al. [1994], Dib et al. [1996], and Wang et al. [1996].

been described before [Bertini et al., 1992]. Retinopathy and PME are major clinical findings in the ceroid-lipofuscinoses (all autosomal recessive traits). This group of storage disease was ruled out in this family on the basis of normal neuropathological study and ultra-structural analysis of skin biopsy.

The two-point lod scores obtained in this study were lower than the canonical threshhold value of 2 for a candidate chromosome. Nevertheless, multipoint analysis data demonstrated that the locus involved in this severe X-linked disorder maps to the distal short arm in Xp22.33-pter, at the boundary or within the

TABLE I. Pairwise Lod Scores Between Xp22.3 Markers and the X-linked Disease Locus

		Z (lod score) at recombination frequencies							
Locus	Location	0.0	0.001	0.01	0.05	0.1	0.2	0.3	0.4
DYS403	Xp22.33	**1.16**	1.16	1.15	1.10	1.02	0.83	0.60	0.32
DXS1233	Xp22.33	**0.96**	0.95	0.93	0.79	0.61	0.23	−0.09	−0.15
DXS7099	Xp22.33	**1.40**	1.39	1.36	1.23	1.06	0.70	0.35	0.09
DXS1228	Xp22.33	0.19	0.19	0.19	0.19	0.18	0.16	0.13	0.07
DXS7100	Xp22.33	**1.64**	1.64	1.60	1.43	1.21	0.74	0.28	0.01
DXS7107	Xp22.33	**0.93**	0.92	0.91	0.87	0.80	0.64	0.45	0.23
DXS1060	Xp22.33	−inf	−0.82	0.15	0.71	**0.84**	0.79	0.59	0.32
DXS996	Xp22.32	−inf	−0.68	0.29	0.85	**0.98**	0.91	0.69	0.38
DXS1223	Xp22.32	−inf	−1.42	−0.44	0.18	0.37	0.45	0.38	0.22

pseudoautosomal region, in a 10 cM interval between DXS1060 and telomere (Fig. 2).

In this interval, a cluster of sulfatase genes on Xp22.3 was identified recently by Ballabio's group [Franco at al., 1995]; genes different from the one involved in chondrodysplasia punctata are potential candidates for this disease.

However, in an exhaustive review, Schaefer et al. [1993] studied a deletion panel involving the distal short arm of the X chromosome, using markers that span about 30 megabases of this region; they described several cases of interstitial and terminal deletions that extend to the markers found to be linked to the locus of the present X-linked condition. Nevertheless, the clinical findings in patients with those interstitial and even terminal deletions do not fit with the severe neurological disorder exhibited by the present family. Thus, two hypotheses could explain this apparent discrepancy:

First, instead of mutations such as deletions that lead to a loss of function, one can suggest a mutation that leads to gain of function such as dynamic CAG triplet repeat amplification and would produce a different effect, as it was already described for the X-linked androgen receptor gene. Indeed, two differents phenotypes involving this gene have been observed: Kennedy disease, a progressive neurological disorder associated with CAG amplification [La Spada et al., 1991] and point mutations or deletions which are responsible for testicular feminization [Marcelli et al., 1991].

Second, the gene (or the genes) involved in the phenotype described in this family could actually be distal with respect to the deletions previously described by Schaefer et al. [1993]. The most telomeric marker used by these authors to define the extent of these "terminal deletions" was PABX which is at the centromeric boundary of the pseudoautosomal region (Fig. 2). Therefore these deletions could not be considered as effective terminal deletions as long as some more telomeric markers are not tested. In the present study, the marker DYS403 which did not show any recombination with the disease, was mapped within the pseudoautosomal region [Dib et al., 1996] and we expect that investigations using further subtelomeric markers as described by Flint et al. [1996] and Dib et al. [1996] will confirm the localization of this disease within the pseudoautosomal region (Fig. 2).

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